

NICEATM

*National Toxicology Program Interagency
Center for the Evaluation of Alternative
Toxicological Methods*

ICCVAM

*Interagency Coordinating Committee on
the Validation of Alternative Methods*



NICEATM-ICCVAM Update

William S. Stokes, DVM, DACLAM
RADM, U.S. Public Health Service
Executive Director, ICCVAM
Director, NICEATM

Meeting of the Scientific Advisory Committee on
Alternative Toxicological Methods

June 16-17, 2011

Arlington, VA



Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

Agency for Toxic Substances and Disease Registry

* Moiz Mumtaz, PhD
Edward Murray, PhD
Eric J. Sampson, PhD

Consumer Product Safety Commission

+ Joanna Matheson, PhD, Vice-Chair
+ Kristina Hattelid, PhD

Department of Agriculture

* Jodie Kulpa-Eddy, DVM Chair
+ Elizabeth Goldentyer, DVM

Department of Defense

* David Honey, PhD
+ Terry Besch, DVM, DACLAM, DACVPM
+ Patty Decot

Department of Energy

* Michael Kuperberg, PhD

Department of the Interior

* Barnett A. Rattner, PhD

Department of Transportation

+ Steve Hwang, PhD

* Principal Agency Representative

+ Alternate Principal Agency Representative

Environmental Protection Agency

Office of Chemical Safety and Pollution Prevention

* John R. Fowle III, PhD, DABT
+ Vicki Dellarco, PhD
+ Tina Levine, PhD
Christine Augustyniak, PhD
Deborah McCall

National Cancer Institute

* T. Kevin Howcroft, PhD
+ Chand Khanna, DVM, PhD

National Institute for Occupational Safety and Health

* Paul Nicolaysen, VMD

National Institute of Environmental Health Sciences

* William S. Stokes, DVM, DACLAM
+ Warren Casey, PhD, DABT
Rajendra S. Chhabra, PhD, DABT
Jerrold J. Heindel, PhD

National Institutes of Health

* Margaret D. Snyder, PhD

National Library of Medicine

* Pertti Hakkinen, PhD
+ Jeanne Goshorn, MS

Occupational Safety and Health Administration

* Surender Ahir, PhD

Food and Drug Administration

Office of the Commissioner

* Suzanne Fitzpatrick, PhD, DABT

Center for Biologics Evaluation and Research

Ying Huang, PhD
Richard McFarland, PhD, MD

Center for Drug Evaluation and Research

+ Abigail C. Jacobs, PhD
Paul C. Brown, PhD

Center for Devices and Radiological Health

Vasant Malshet, PhD, DABT

Center for Food Safety and Nutrition

David G. Hattan, PhD
Diego Rua, PhD

Center for Veterinary Medicine

M. Cecilia Aguila, DVM

Li You, PhD

National Center for Toxicological Research

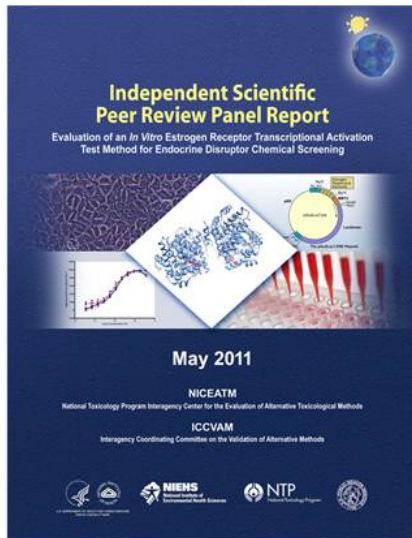
Paul Howard, PhD
Donna Mendrick, PhD



Updates

- Endocrine Disruptor Screening Test Methods
- Biologics Safety and Potency Testing
- Allergic Contact Dermatitis Safety Assessments
- Ocular Safety Assessments
- Acute Systemic Toxicity
- Genetic Toxicity Test Methods
- Test Method Nominations
- International Cooperation on Alternative Test Methods
- Outreach Activities
- Regulatory Acceptance of Alternative Methods

Endocrine Disruptor Chemical Screening Methods



- LUMI-CELL® stably-transfected transcriptional activation assay
 - Human ovarian carcinoma cell line
 - ER agonist and antagonist protocols
 - International validation completed, 2010
 - International Peer review meeting: March 29-30, 2011
 - **Agenda item today**
- MCF-7 Cell Proliferation Assay, CertiChem, Inc.
 - Human breast adenocarcinoma cell line
 - ER agonist and antagonist protocols
 - International validation completed, March 2011
- ICCVAM Interagency Endocrine Disruptor Working Group
 - ECVAM, JaCVAM, KoCVAM liaisons



NICEATM-ICCVAM International Workshop: Vaccine Potency and Safety Testing



- September 14-16, 2010
 - Co-organizers: ECVAM, JaCVAM, Health Canada
 - Nearly 200 scientists, 13 countries
- Human and veterinary vaccines
 - 5-year Plan priority
- Objectives
 - Status of 3Rs alternatives
 - Identify priority vaccines for future progress
 - Identify priority R&D, validation needs
 - Identify ways to increase use, international harmonization
- ICCVAM Interagency Biologics Working Group
- Proceedings, Fall 2011
 - *Procedia in Vaccinology*
- Agenda item tomorrow



International Workshop on Alternative Methods for Rabies Vaccine Potency Testing



- USDA National Centers for Animal Health; Ames, Iowa
 - October 11-13, 2011
- Organized by NICEATM-ICCVAM with ICATM partners
- Highest priority from September 2010 workshop
- Address both human and veterinary rabies vaccines
- International scientific experts, regulatory authorities, industry
- Agenda item tomorrow

Allergic Contact Dermatitis (ACD) Safety Assessment Methods: ICCVAM Evaluations



- Agency acceptance of ICCVAM recommendations in March 2011
 - 2 nonradioactive LLNA versions
 - Updated LLNA applicability domain
- Usefulness of LLNA for potency categorization
 - Evaluation completed, 2010
 - Transmittal to federal agencies in progress
- ICCVAM Interagency Immunotoxicity Working Group
 - ICATM liaisons
- Regulatory acceptance updates

Allergic Contact Dermatitis Safety Assessment Methods: Other ICATM Collaborations

- *In vitro/in chemico* methods
 - Direct peptide reactivity assay (DPRA)
 - Myeloid U937 skin sensitization test (MUSST)
 - Human cell line activation test (h-CLAT)
 - NICEATM-ICCVAM on Validation Management Team for ECVAM-led ongoing study
- KeratinoSens
 - uses HaCaT cells and is based on Nrf2-Keap1-ARE regulatory pathway
 - Upcoming ECVAM-led peer review of DPRA and KeratinoSens; NICEATM-ICCVAM nominations for peer review
- JaCVAM to begin validation study in the fall for an *in vitro* skin sensitization assay
 - Uses IL-8 and G3PDH reporter constructs in THP-1 cells
 - NICEATM-ICCVAM participation on Validation Management Team

Ocular Safety Testing Methods: ICCVAM Evaluation and Recommendations



- Agency acceptance/endorsement of ICCVAM recommendations in March 2011
- Recommendations provided for 10 alternative test methods and strategies
 - Routine use of analgesics, topical anesthetics, and humane endpoints
 - Low volume eye test
 - *In vitro* test methods and strategy
- Regulatory acceptance updates

ICCVAM. 2010. NIH Publication No. 10-7553A. RTP, NC NIEHS.
<http://iccvam.niehs.nih.gov/methods/ocudocs/MidMod-TMER.htm>

Ocular Safety Testing Methods:

Other International and ICATM Activities

- OECD
 - Updating of TG 405 to include additional humane endpoints and routine use of analgesics and anesthetics
 - Draft TG circulated to member countries, June 6, 2011
 - Draft Guidance Document for using histopathology in ocular safety testing-endorsed in April by NCs
- ICATM validation studies
 - Short time exposure (STE) test using rabbit corneal epithelial cell line (SIRC cells)
 - JaCVAM validation study completed
 - NICEATM-ICCVAM to coordinate independent peer review
 - EpiOcular and SkinEthic
 - Ongoing ECVAM-led validation study
 - NICEATM and ICCVAM VMG liaisons
- Related studies
 - NIH-FDA Regulatory Science Grant: RoBatt: Replacement ocular battery using existing *in vitro* test methods for eye injury assessment (MB Research Labs)

Ocular Safety Testing: Using Fewer Animals to Identify Chemical Eye Hazards

- CPSC request
 - Propose criteria for hazard classification for a 3-animal test equivalent to current requirements (6 to 18 animals) in FHSA regulations
- Three hazard criteria compared:
 - Current
 - $\geq 1/3$ positive animals
 - $\geq 2/3$ positive animals
- Updated analysis:
 - Review of 481 studies (6-animal tests)
 - Estimated over- and underprediction rates
- Results:
 - Criterion of $\geq 1/3$ positive animals provides same or greater level of eye hazard labeling as current FHSA requirements
 - New criterion will reduce animal use 50-83%
- Recommendations in progress

Acute Systemic Toxicity Activities

- *In vitro* model for human hepatic metabolism and toxicity
 - NICEATM-ICCVAM participating on ECVAM Validation Management Team
- Development of acute dermal toxicity Up-and-Down Procedure
 - NICEATM compiling acute dermal and oral toxicity data for simulation studies
 - 2127 acute dermal toxicity studies
 - 429 substances with acute oral toxicity data
- Using 3T3 NRU cytotoxicity assays to classify EU “non-toxic” substances (oral LD₅₀ > 2000 mg/kg) without animal testing
 - ECVAM validation study completed; upcoming peer review
 - NICEATM nominations for peer review working group

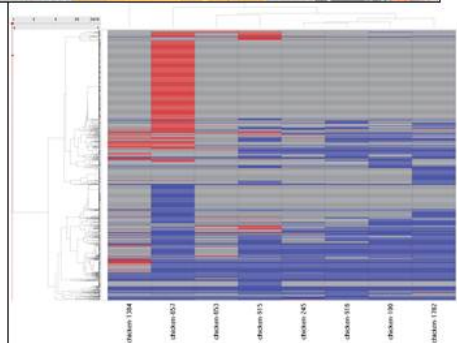
Genetic Toxicity Test Method Activities

- ***In vivo* and *in vitro* Comet assays**
 - JaCVAM-led international validation studies
 - Phase IV *in vivo* study completed Oct 2010
 - Validation report expected in 2011
 - Phase III *in vitro* study ongoing
 - ICCVAM comments on proposed study plans, protocols, and reference substances
- **Cell transformation assays**
 - Bhas – JaCVAM study completed
 - SHE and Balb/c 3T3 - ECVAM peer review Feb 2011
- **ICCVAM Interagency Genetic Toxicity Working Group**
 - ICATM liaisons

Recent Test Method Nominations

- An *in vitro* pyrogen test method for assessing non-endotoxin pyrogens
- *In vitro* assays for the detection and quantification of botulinum neurotoxins
- Agenda items this afternoon

Developing Future Test Methods: High Throughput *In Vitro* Screening



- NIH (NIEHS and NHGRI), EPA, and FDA collaborating to use the NIH Chemical Genomics Center: "Tox 21"
 - Robotic quantitative high-throughput *in vitro* screening (HTS)
 - ToxCast™: 600 assays
- Using 10,000 chemicals to identify toxicity pathways
- NICEATM-ICCVAM
 - >900 ICCVAM reference chemicals nominated for inclusion
 - NICEATM-ICCVAM nomination of *in vitro* assays for HTS; Assay selection
 - Will monitor results for *in vitro* test methods with pathway-based predictive biomarkers

Outreach Activities: Workshops on Best Practices for Regulatory Safety Testing



ICCVAM Workshop Series on Best Practices for Regulatory Safety Testing:

Two one-day workshops on available alternative methods that evaluate hazard potential of chemicals and products, minimize animal use, and avoid animal pain and distress.

January 19, 2011: Assessing the Potential for Chemically Induced Eye Injuries

January 20, 2011: Assessing the Potential for Chemically Induced Allergic Contact Dermatitis

William H. Natcher Conference Center
National Institutes of Health — Bethesda, MD, USA

The workshop is open to the public with no registration fee.
For more information and to register, please contact NICEATM:
website: <http://iccvam.niehs.nih.gov>
phone: 919-541-2384 email: niceatm@niehs.nih.gov



Organized by: NICEATM - National Toxicology Program Interagency Center
for the Evaluation of Alternative Toxicological Methods
ICCVAM - Interagency Coordinating Committee on the Validation of Alternative Methods

ICCVAM Agencies:

• Agency for Toxic Substances and Disease Registry
• Consumer Product Safety Commission
• Department of Agriculture
• Department of Defense
• Department of Education
• Department of Energy
• Department of Health and Human Services
• Environmental Protection Agency
• Food and Drug Administration
• National Aeronautics and Space Administration
• National Endowment for the Arts
• National Endowment for the Humanities
• National Science Foundation
• National Security Agency
• National Security Council
• National Toxicology Program
• Small Business Administration
• Social Security Administration
• State Department
• Supreme Court
• Transportation Department
• Veterans Affairs Department
• White House



- NIH Natcher Center
- January 19, 2011: Ocular safety testing
 - 77 participants
- January 20, 2011: Allergic contact dermatitis (ACD) hazard testing
 - 76 participants
- Agenda item this afternoon

Outreach Activities: 2011 Annual Society of Toxicology Meeting

- March 6-10, 2011, Washington, DC
- 8 posters
- ICATM Informational Session:
*The International Cooperation on
Alternative Test Methods: Translating
Science to Provide Improved Public
Health Safety Assessment Methods*



Available at: <http://iccvam.niehs.nih.gov/meetings/SOT11/sotablst.htm>

Outreach: Eighth World Congress on Alternatives and Animal Use in the Life Sciences

- August 21-25, 2011
- Montreal, Canada
- 11 NICEATM-ICCVAM presentations



International Cooperation on Alternative Test Methods (ICATM)

■ Memorandum of Cooperation

- 1st Modification to add KoCVAM
 - Signed March 8, 2011
- Original Signed: April 27, 2009

■ Signatories

- **Dr. Linda Birnbaum**,
Director, NIEHS and NTP, USA
- **Dr. Masahiro Nishijima**,
Director General, National Institute of Health Sciences, Japan
- **Dr. Elke Anklaam**, Director, Institute of Consumer Protection and Health, Joint Research Centre, European Commission
- **Dr. David Blakey**, Director, Health and Safety Bureau, Health Canada
- **Dr. Seung Hee Kim**, Director General, National Institute of Food and Drug Safety Evaluation, Korea Food and Drug Administration



NEW



ICATM Organizations

ICATM is a **voluntary** international cooperation of national organizations: Canada, the European Union, Japan, South Korea, and the United States.



**Health
Canada**



**NICEATM-
ICCVAM**



ECVAM



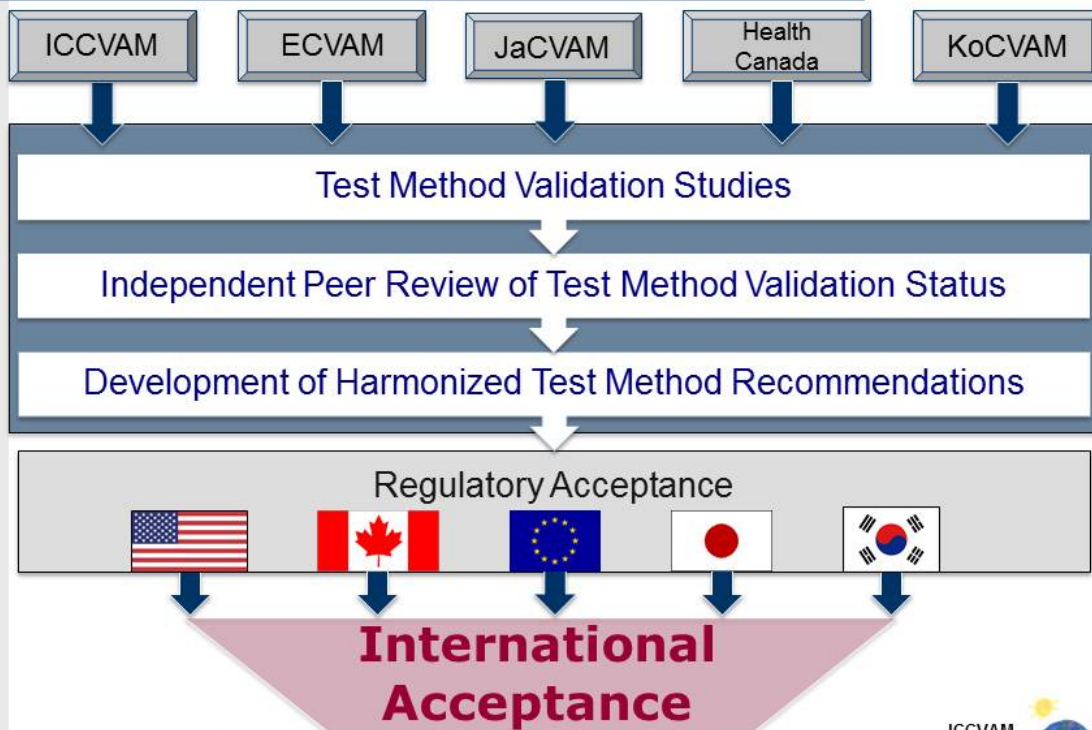
KoCVAM



JaCVAM



ICATM Cooperation



Acknowledgements: NICEATM

NIEHS

William S. Stokes, DVM, DACLAM	<i>Director</i>
Warren Casey, PhD, DABT	<i>Deputy Director</i>
Debbie McCarley	<i>Special Assistant</i>

Center Support Contract (ILS, Inc.)

David Allen, PhD, Principal Investigator	Steven Morefield, MD, Project Manager
Thomas Burns, MS	Patricia Ceger, MS
Jonathan Hamm, PhD	Nelson Johnson
Brett Jones, PhD	Elizabeth Lipscomb, PhD
Linda Litchfield	Anna Lee Mosley
Michael Paris	Catherine Sprankle
Frank Stack	Judy Strickland, PhD, DABT
Linda Wilson	James Truax, MA

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Regulatory Acceptance of ICCVAM-Recommended Alternative Test Methods

William S. Stokes, DVM, DACLAM
RADM, U.S. Public Health Service
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Director, NICEATM

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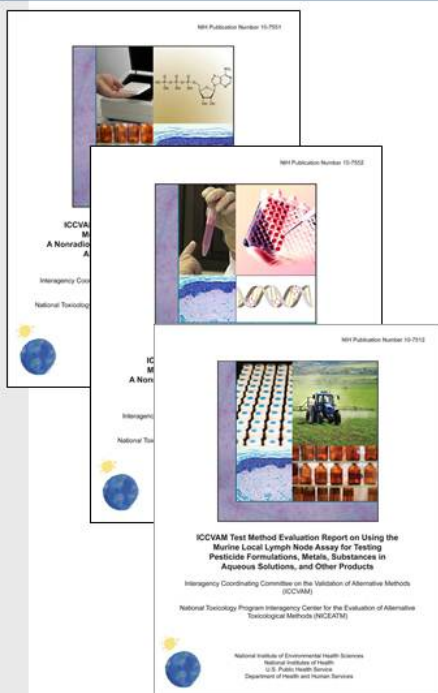
Arlington, VA



Outline

- U.S. Regulatory Acceptance of Alternative Methods
 - Allergic contact dermatitis (ACD) safety assessments
 - Eye safety assessments
- International Regulatory Acceptance
 - ACD safety assessments
 - Acute oral systemic toxicity safety assessments
 - Dermal irritation safety assessments
 - Genetic toxicity safety assessments

Federal Agency Acceptance: ICCVAM Recommendations for Alternative Methods for Allergic Contact Dermatitis



- ICCVAM recommendations transmitted to Federal agencies on June 12, 2010:
 - **Nonradioactive LLNA Versions**
 - LLNA: DA (Diacel-ATP)
 - LLNA: BrdU-ELISA
 - 3Rs benefits of LLNA without using radioactivity
 - **Updated Applicability Domain of the LLNA**
 - Pesticide formulations and other products
 - Metals, except nickel
 - Substances in aqueous solutions
 - Other substances/products unless physicochemical properties interfere with the ability of the LLNA to detect sensitizers
 - Expands 3Rs benefits of LLNA

LLNA Advantages

	GPMT¹	LLNA
■ Time to perform:	22+ days	7 days
■ Number of animals:	30	12-20
■ Dermatitis induced:	Yes	No
■ Adjuvant required:	Yes	No

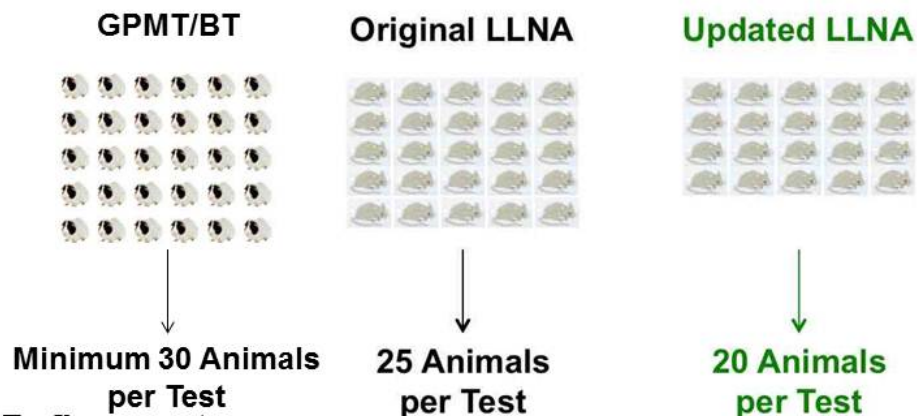


□ Advantages over guinea pig test methods

- Elimination of potential pain and distress
- 33-60% fewer animals
- 60% reduction in time to perform
- Dose-response information

Updated ICCVAM LLNA Protocol: Reduction and Refinement

- 20% **reduction** in animal numbers (4 vs. 5/group)

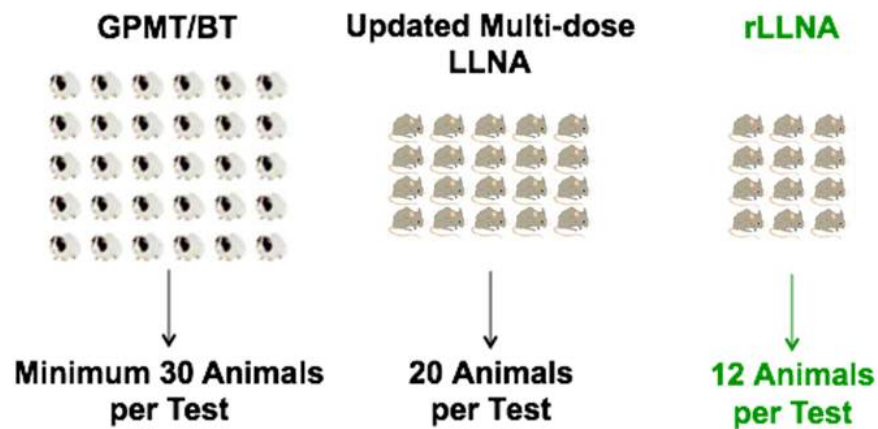


- **Refinement**

- Avoids pain and distress associated with guinea pig tests:
 - Avoids the elicitation phase involving pruritis, erythema, edema
 - Avoids the need for irritating adjuvants (i.e., GPMT)

Reduced LLNA (rLLNA)

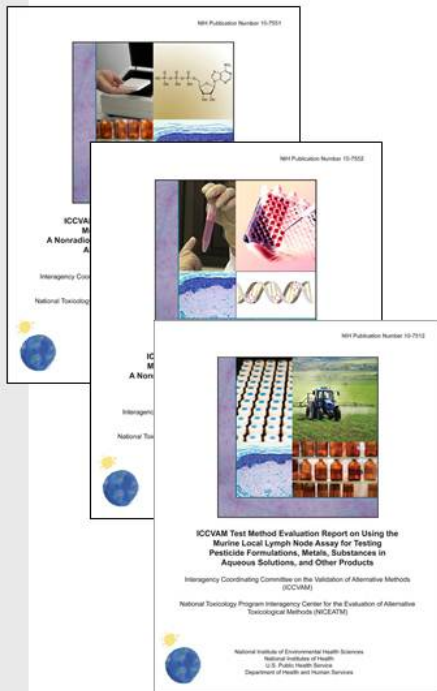
- Modification of multi-dose LLNA that uses fewer animals to assess ACD hazard potential
 - For each test substance, rLLNA tests only the highest dose vs. at least three doses for LLNA
 - rLLNA reduces animal number by 40% for each test vs. multi-dose LLNA



Abbreviations: GPMT/BT = guinea pig maximization test/Buehler test



Alternative Methods for ACD: Federal Agency Responses to ICCVAM Recommendations



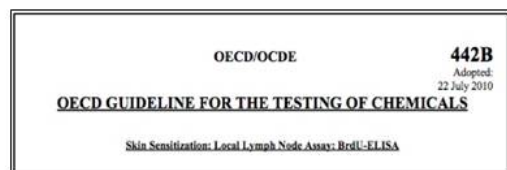
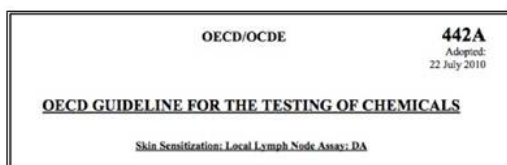
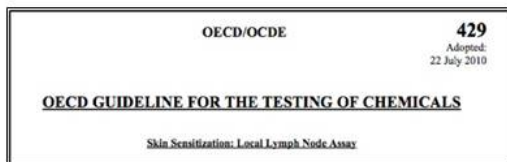
■ Agency responses

- Agencies agreed with ICCVAM recommendations where applicable to their agency
- FDA noted limitations of LLNA-DA
 - Potential for false positives with borderline weak positive responses between an SI of 1.8 and 2.5
 - Might not be appropriate for testing substances that affect ATP levels, or the accurate measurement of intracellular ATP

- Available at:

<http://iccvam.niehs.nih.gov/methods/immunotox/llna.htm>

New and Updated LLNA-based Test Methods: International Acceptance



■ Updated OECD Test Guideline 429 for the LLNA

- Updated LLNA test method protocol procedures
- rLLNA procedure
- LLNA performance standards
- Use of the LLNA for testing pesticides formulations, metals, and aqueous solutions

■ Test Guideline 442A – LLNA: DA (Diacel-ATP)

■ Test Guideline 442B – LLNA: BrdU-ELISA

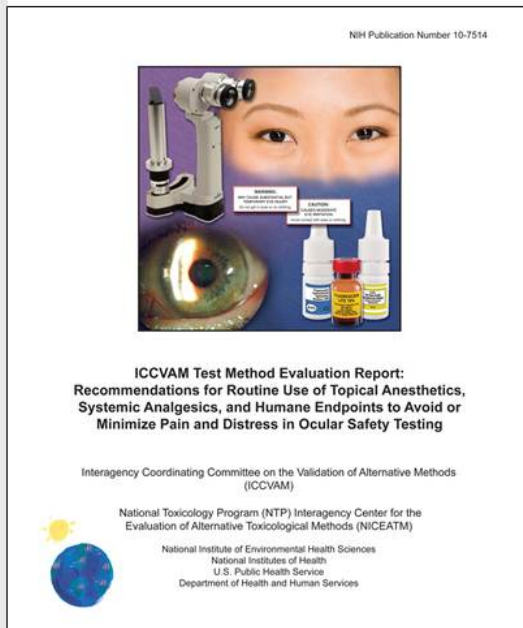
■ All 3 available at:

<http://www.oecd-ilibrary.org>

<http://iccvam.niehs.nih.gov/methods/immunotox/llna.htm>

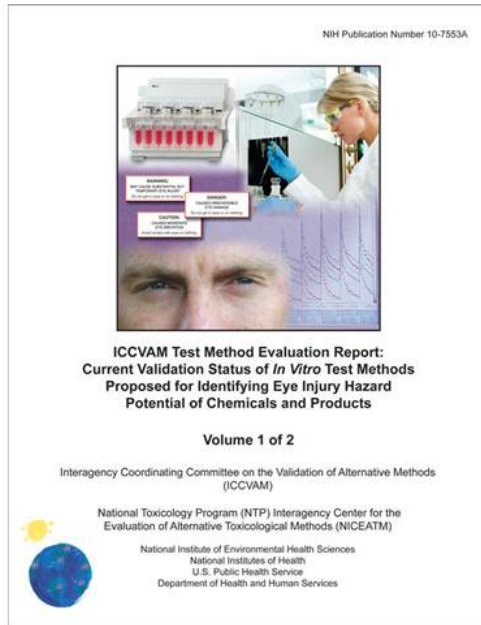


ICCVAM Recommendations to Refine the Ocular Safety Test: Federal Agency Acceptance



- Routine use of analgesics, topical anesthetics, and humane endpoints for required in vivo ocular safety testing
 - Expected to avoid most if not all pain and distress for eye safety testing
 - Proposal to update to OECD eye testing guideline (TG 405)
 - 2012 adoption expected
- <http://iccvam.niehs.nih.gov/methods/ocutox/pretreat.htm>

ICCVAM Recommendations for *In Vitro* Alternative Methods for Eye Safety Assessments: Federal Agency Acceptance



- In vitro method to ID substances not requiring ocular hazard labeling
 - Cytosensor™ recommended for limited types of substances
 - First *in vitro* test method recommended for confirming lack of hazard
- BCOP, ICE, IRE, HET-CAM
 - insufficient predictivity for non-severe effects to recommend at this time
 - additional optimization studies recommended
- <http://iccvam.niehs.nih.gov/methods/ocutox/MildMod.htm>

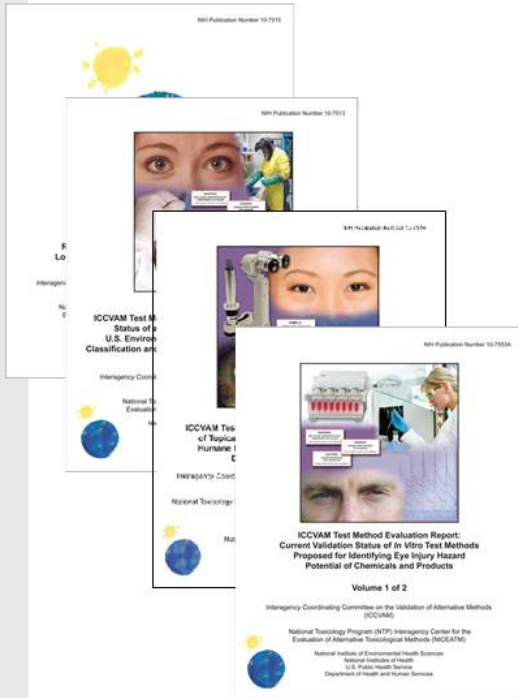
ICCVAM Recommendations for Alternative Methods/Strategies for Eye Safety Assessments: Federal Agency Acceptance



- In vitro testing strategy to assess eye irritation potential for antimicrobial cleaning products for U.S. EPA hazard classification
 - CM, BCOP, EpiOcular
 - Promising, but small database; additional studies recommended
 - EPA Pilot study encouraging data submission
 - <http://iccvam.niehs.nih.gov/methods/ocutox/AMCP.htm>
- Low volume eye test: recommendation to discontinue use
 - <http://iccvam.niehs.nih.gov/methods/ocutox/LVET.htm>

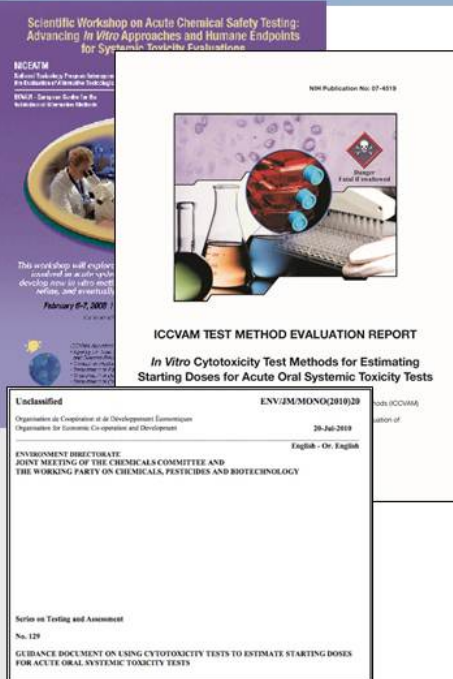


Alternative Methods for Eye Safety Assessments: Federal Agency Responses to ICCVAM Recommendations



- Agency responses provided per Public Law 106-545: March, 2011
- Agencies agreed with ICCVAM recommendations where applicable to their agency
- Responses available at: <http://iccvam.niehs.nih.gov/methods/ocutox/Transmit-2010.htm>

International Acceptance: *In Vitro* Methods for Acute Systemic Toxicity Testing



<http://iccvam.niehs.nih.gov/methods/acute/tox/acute/tox>

■ OECD Guidance Document No. 129

- *Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests*
- Published July 2010

■ Based on 2008 ICCVAM Evaluation Report

- *In vitro* methods can reduce animal use up to 50% per test
- Should always be considered before using animals for acute oral toxicity, use where appropriate

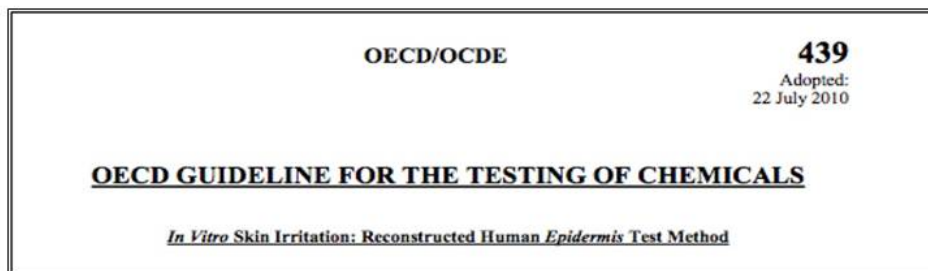
■ Endorsed by Federal agencies in 2008

■ ICCVAM interagency Acute Toxicity Working Group

- ECVAM and JaCVAM liaisons

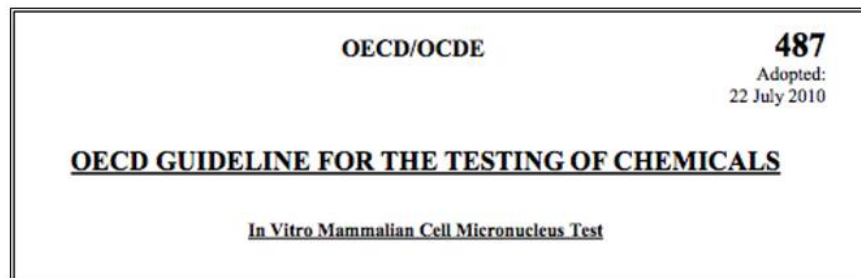
Dermal Irritation Test Methods: International Acceptance

- OECD Test Guideline 439: In Vitro Skin Irritation – Reconstructed Human Epidermis Test Method
 - EpiDerm™ Assay
 - EPISKIN™ Assay
 - SkinEthic RHE Assay
- OECD Formal Adoption: July 22, 2010
- Available at: <http://www.oecd-ilibrary.org>



Genetic Toxicity Test Methods: International Acceptance

- OECD Test Guideline 487: *In Vitro* Micronucleus Assay
- OECD Formal Adoption: July 22, 2010
- Available at: <http://www.oecd-ilibrary.org>



Thank you for your attention.

Questions?

Dr. William Stokes
stokes@niehs.nih.gov

<http://iccvam.niehs.nih.gov/>

Questions for SACATM

1. Regarding the alternative testing methods recently accepted for assessing the ocular safety and allergic contact dermatitis hazard potential of chemicals and products, how might Federal agencies promote and encourage the use of these alternative methods for the purpose of complying with Federal testing statutes, regulations, guidelines, and recommendations?
2. Do you have suggestions on ways to successfully implement consideration and use of these methods by the regulated community? How might Federal agencies, NICEATM and/or ICCVAM help facilitate this process?